1: J Biol Chem 1992 Jul 25;267 (21):14799-803

Multiple forms of ubiquitin-activating enzyme E1 from wheat. Identification of an essential cysteine by in vitro mutagenesis.

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Ubiquitin-activating enzyme, E1, directs the ATP-dependent formation of a thiol ester linkage between itself and ubiquitin. The energy in this bond is ultimately used to attach ubiquitin to various intracellular proteins. We previously reported the isolation of multiple E1s from wheat and the characterization of a cDNA encoding this protein (UBA1). We now report the derived amino acid sequence of two additional members of this gene family (UBA2 and UBA3). Whereas the amino acid sequence of UBA2 is nearly identical to UBA1, the sequence of UBA3 is significantly different. Nevertheless, the protein encoded by UBA3 catalyzes the ATP-dependent activation of ubiquitin in vitro. Comparison of derived amino acid of genes encoding E1 from plant, yeast, and animal tissues revealed 5 conserved cysteine residues, with one potentially involved in thiol ester bond formation. To identify this essential residue, codons corresponding to each of the 5 cysteines in UBA1 were individually altered using site-directed mutagenesis. The mutagenized enzymes were expressed in Escherichia coli and assayed for their ability to activate ubiquitin. Only substitution of the cysteine at position 626 abolishes E1 activity, suggesting that this residue forms the thiol ester linkage with ubiquitin.

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Two distinct ubiquitin-proteolysis pathways in the fission yeast cell cycle.

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The SCF complex (Skp1-Cullin-1-F-box) and the APC/cyclosome (anaphase-promoting complex) are two ubiquitin ligases that play a crucial role in eukaryotic cell cycle control. In fission yeast F-box/WD-repeat proteins Pop1 and Pop2, components of SCF are required for cell-cycle-dependent degradation of the cyclin-dependent kinase (CDK) inhibitor Rum1 and the S-phase regulator Cdc18. Accumulation of these proteins in pop1 and pop2 mutants leads to re-replication and defects in sexual differentiation. Despite structural and functional similarities, Pop1 and Pop2 are not redundant homologues. Instead, these two proteins form heterodimers as well as homodimers, such that three distinct complexes, namely SCFPop1/Pop1, SCFPop1/Pop2 and SCFPop2/Pop2, appear to exist in the cell. The APC/cyclosome is responsible for inactivation of CDK/cyclins through the degradation of B-type cyclins. We have identified two novel components or regulators of this complex, called Apc10 and Ste9, which are evolutionarily highly conserved. Apc10 (and Ste9), together with Rum1, are required for the establishment of and progression through the G1 phase in fission yeast. We propose that dual downregulation of CDK, one via the APC/cyclosome and the other via the CDK inhibitor, is a universal mechanism that is used to arrest the cell cycle at G1.

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